Recombinant expression and studies on DCL-1, receptor of dendritic cells

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ABSTRACT

Dendritic cells, which could be found in large numbers in many tissues, exprime C-type lectine receptor DCL-1 (in CD nomenclature CD302) on their surface. This molecule belongs to the class one transmembrane proteins. N-terminal sequence a C-type lectin-like domain (CTLD) it has on the extracellular side. Whereas the C-terminus, which contains potential phosphorylation site and signals for intracellular transport, is in the cytosol. As the sequence of this protein is highly conserved through all mammalian species, it is likely to play an important role in the immune system. Despite this fact, the DCL-1 molecule is still poorly investigated.

This work deals with the extracellular part – especcially with the CTLD containing part – of the DCL-1 receptor. To learn more about this molecule, we attempted its recombinant expression in bacteria. We used pET-30a(+) based vector pDCL1E, and bacterial cells *E. coli* BL21 (DE3) Gold, which produced the target protein in the form of inclusion bodies into the cytoplasm. Therefore conditions for *in vitro* renaturation were optimalized, and it was prooved that the protein had native conformation necessary for research into its structure and function.

In the next step the molecule was labeled with fluorescent dyes NHS-Fluorescein and Alexa Fluor 488. The protein labeled with Alexa Fluor 488 was sent – for examination of protein-carbohydrate interactions using wide arrange of potential oligosaccharide ligands – to Consorcium for Functional Glycomics (CFG). In our laboratory we also accomplished some binding tests with the ^{125}I labeled protein molecule on carbohydrates. Investigations still underway should contribute to our understanding of the nature of DCL-1 ligands, and thus help to reveal its biological functions.

(In Czech)